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Microbiological quality of typical foods served in Thai restaurants in Vienna, Austria

Mikrobiologische Beschaffenheit von typischen Gerichten in Wiener Thai Restaurants

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Summary

The microbiological quality, pH and serving size of typical meals served in 18 Thai restaurants in Vienna were studied. During the summer and winter season, one ready-to-eat food portion was sampled per restaurant and per food category ("A": raw or insufficient heat treatment; "B": heat treated main dish garnished/mixed with raw side-dishes; "C": thorough heat treatment, served alone; total number = 108). In addition, – where possible – some typical side dishes were sampled (n = 25). The average weight of main dishes varied dependent on category from 360–510 g. The lowest pH was observed in group "A" (average 4.5), most probably because of the addition of acids via lime juice. Expectedly, group "A" had significantly higher total aerobic counts (6.1 ± 0.8 log cfu/g) than group "B" (4.5 ± 1.7 log cfu/g) and "C" (2.6 ± 0.9 log cfu/g). In side-dishes, average total aerobic counts were higher in raw vegetables (4.9 log cfu/g) than in rice (2.6 log cfu/g). With respect to hygiene indicator bacteria, there was a clear association between presumably insufficient heat treatment and/or the likelihood of cross-contamination (groups "A", "B") on the one hand and the presence of *E. coli* and Enterobacteriaceae on the other. All 133 samples tested negative for *Salmonella* spp., *Listeria monocytogenes* and *Campylobacter* spp. (25 g aliquots). In groups "A" and "B" the warning limits for total aerobic count and Enterobacteriaceae established by the German Society for Microbiology and Hygiene were exceeded in 30–60 % of the samples.

For food classification we used an empirical scheme taking into account heat treatment and the likelihood of cross-contamination, assuming that these two factors would be associated with the risk of presence of pathogenic bacteria in the ready-to-eat food portion. This empirical classification was well reflected in total aerobic counts. As regards pathogens, the usefulness of such a classification scheme could not be evaluated due to the low frequency of pathogens. This indicates that on this issue further studies should be conducted.

Keywords: Thai food, ready-to-eat food, serving size, microflora, pH, consumer exposure

Zusammenfassung

In dieser Arbeit wurden die mikrobiologische Beschaffenheit, pH Werte und die Portionsgrößen von typischen Gerichten aus 18 thailändischen Restaurants in Wien untersucht. Dabei wurde je Restaurant und Lebensmittelkategorie („A": rohe oder nicht durcherhitzte Hauptmahlzeiten „B": durcherhitzte Hauptmahlzeiten, die mit rohen Komponenten garniert bzw. gemischt wurden; „C": durcherhitzte Hauptspeisen, die ohne rohe Beilage serviert wurden, insgesamt n = 108) in der Sommer- und der Wintersaison jeweils eine verzehrfertige Hauptspeise beprobt. Soweit möglich, wurden auch noch typische Beilagen beprobt (n = 25). Das durchschnittliche Portionsgewicht betrug je nach Lebensmittekategorie 360–510 g. Kategorie „A"-Lebensmittel wiesen dabei die niedrigsten pH Werte auf (Mittelwert 4,5), was sich z. T. durch Säurezusatz per Limettensaft erklären lässt. Wie zu erwarten war, wies Gruppe „A" signifikant höhere aerobe mesophile Gesamtkeimzahlen auf (6,1 ± 0,8 $\log_{10}$ kbE/g) als die Gruppen „B" (4,5 ± 1,7 $\log_{10}$ kbE/g) und „C" (2,6 ± 0,9 $\log_{10}$ kbE/g). Bei den Beilagen war die aerobe mesophile Keimzahl in rohem Gemüse höher (4,9 $\log_{10}$ kbE/g) als in Reis (2,6 $\log_{10}$ kbE/g). Hinsichtlich Hygieneindikatorbakterien ergab sich ein klarer Zusammenhang zwischen mutmaßlicher Untererhitzung und möglicher Kreuzkontamination (Gruppen „A", „B") und dem Nachweis von *E. coli* und Enterobacteriaceae. *Salmonella* spp., *Listeria monocytogenes* und *Campylobacter* spp. konnten in keiner der 133 Proben nachgewiesen werden (25 g Probenmenge). In den Gruppen „A" und „B" wurden die Warnwerte der DGHM für aerobe mesophile Keimzahl und Enterobacteriaceae bei 30–60 % der Proben überschritten.

Die in dieser Arbeit angewendete Lebensmittelklassifizierung erfolgte rein empirisch auf Grundlage des Erhitzungsregimes und der Möglichkeit der Kreuzkontamination, mit der Überlegung, daß die beiden genannten Faktoren das Risiko des Vorkommens pathogener Bakterien in der verzehrfertigen Portion erhöhen. Diese Klassifizierung spiegelte sich zwar gut in der aeroben mesophilen Keimzahl wieder, bei den pathogenen Bakterien war aber die Nachweishäufigkeit zu gering, um den Nutzen des Klassifizierungsschemas beurteilen zu können. Daher sollten weiterführende Studien durchgeführt werden.

Schlüsselwörter: Thailändische Gerichte, verzehrfertige Speisen, Portionsgröße, Mikroflora, pH, Exposition des Konsumenten

Introduction

Migration of people has enhanced culture and food in European countries including Austria. For a long time immigrants have primarily been considered to represent a low-skilled and cheap labour force recruited in order to counter the problem of labour shortage in Austria (Castles and Miller, 2003; Mayer, 2010). Official data (Statistik Austria, 2012) registered 23 152 South-East Asians in Austria, with more than half of them living in the capital city, Vienna. Immigrant groups from the Philippines, Thailand, and Vietnam constitute 90.9 % of the South-East Asian population in Vienna. Migrations as well as tourism have increased the demand for "exotic" foods placed on the market in European countries. Whereas data exists on the microbiological condition of exotic food ingredients as well as ready-to-eat foods obtained in specialized shops or via E-commerce (Grabowski and Klein, 2010), there are, with the exception of "fast-food" products (e. g. Omurtag et al., 2012), few reports on ready-to-eat ethnic food served in restaurants.

Thai restaurants represent one of the most popular immigrant businesses of Thai people in Vienna and the major group of consumers are Austrian rather than Thais (Butratana and Trupp, 2011). In 2011, the Department of Export Promotion, of the Ministry of Commerce, Royal Thai Government has registered 34 Thai restaurants in Austria, of which 19 are located in Vienna. This illustrates the popularity and acceptability of ethnic Thai food to Austrian people. Jamal (2003) concluded that consumers of different ethnic backgrounds are skilled navigators to sample the many tastes, themes and sounds of different cultures.

With the influences and mixture from various parts of the world, Thai food is composed of a broad range of food ingredients and is prepared by various cooking methods. Traditional Thai cooking methods include stewing, baking and grilling. Chinese influences were the introduction of frying; stir frying, deep-frying, and Indian curry was adapted by substituting dairy products with coconut milk to make Thai curries. The main components of Thai food are meat, vegetables, herbs and spices and only lightly prepared dishes with strong aromatic components. Thai food is generally served hot, and Thai eating style is usually based on sharing dishes, and only rice is served in individual portions. However, as an adaptation to western eating style, many Thai restaurants offer Thai food as individual dishes.

In Thailand, diarrheal diseases have been a major public health problem for many years. Food is considered as a main route of transmission. There are approximately a million cases of acute diarrhea reported each year. In 2011, the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Heath (2011) reported 100 534 cases of food poisoning. Major agents identified were *Vibrio parahaemolyticus, Salmonella, Staphylococcus* spp., *Clostridium botulinum* and *Clostridium perfringens,* and consumption of microbially contaminated drinking water and food were recorded as the major pathways for diarrheal diseases in Thailand. Diarrheal diseases are usually found in individuals living in poorly sanitized environments and/or those practising poor personal hygiene.

In the European Union, the European Food Safety Authority (2012) reported a total of 5262 food-borne outbreaks for the year 2010 with *Salmonella,* viruses, *Campylobacter* and bacterial toxins (*Bacillus, Clostridium* and *Staphylococcus*) being the main causative agents in these outbreaks whilst main food sources were eggs, mixed or buffet meals and vegetables. Apart from households, the most common settings in 'strong evidence' outbreaks were restaurants/cafes and similar premises (30.8 % of outbreaks, 26.0 % of human cases).

As by definition of EC No 2073/2005 (EC, 2005), ready-to-eat foods comprise food items intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level micro-organisms of concern. Thus, food served in restaurants is supposed to meet the microbial limits. The microbiological condition of Thai food is generally unknown, and also the influence of the quality of raw material such as meat, vegetables, herbs and spices, and of heat processing as well as kitchen hygiene in the various Thai restaurants on the microbiological profile of the ready-to-eat servings.

The aims of the present study were (1) to classify typical Thai ready-to-eat foods according to their potential of allowing hazardous bacterial agents to either survive the preparation process, or being introduced into the final product via cross contamination (derived from the scheme presented by Omurtag et al., 2013); (2) to assess the microbiological condition of such food retailed in Vienna, and to compare it to recommended limits as issued by the German Society for Hygiene and Microbiology (DGHM, 2012); this should serve to estimate if and to what extent consumers are exposed to hazardous bacteria; and finally, (3) to explore if the food classification scheme matches the results from microbiological examination. The classification scheme used in our study could, however, be applied to other groups of ethnic foods and thus, allow comparison between typical foods from different regions or continents.

Materials and Methods

Sampling plan

Sampling sites were defined as restaurants in Vienna offering typical Thai foods. A total of 19 restaurants was identified, most of these clustered in the central districts of the city while two of them were located in the city's periphery, north of the Danube river. Based on general information about Thai cuisine and the menu cards of the restaurants, foods were categorized according to heat treatment and the possibility of cross-contamination during the assembly of the complete meal, according to the scheme recently developed by Omurtag et al. (2013). In brief, well-known Thai dishes were divided into 3 categories ("A"–"C") as follows.

A. Dishes without heat treatment (uncooked) or heat treatment affecting the meat surface only, such as sôm tam (Papaya salad) and yam nûa yaan (grilled beef salad), respectively.
B. Dishes which receive thorough heat treatment, but prior to serving fresh vegetable is either added to the cooked dishes or served as side dish such as khâaw phat (Fried Rice), phat thay (Thai stirred noodle), and laap (Thai minced meat with herbs and spices).
C. Dishes which can be considered as safe according to heat treatment process such as tôm yam, tôm khâa (Thai Soup), kɛɛn khĭaw wăan (Thai green curry), kɛɛn phèt (Thai red curry), and which are not combined with fresh vegetables or unheated side-dishes.

Detailed information on main ingredients and heat treatments are presented in Table 1. Notably, this classification was empirical, i. e. based on the currently widely accepted food preparation techniques, but not involving any measure-

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ments. Considering the characteristics of the Thai cuisine, two additional food categories for side-dishes served separately were studied, i. e. rice ("D") and vegetables served as raw ("E") such as cabbage, cucumber, bean sprout and carrot.

TABLE 1: *Descriptions of main ingredients and heat treatment of food sample from category A, B and C.*

Category	Name	Main ingredients	Heat treatment
A	sôm tam (Papaya salad)	papaya, dry shrimp, yard long bean, chilli, garlic, lime juice, peanut, with or without fermented crab or fermented fish	no heat treatment
	yam nâa yaan (Grilled beef salad)	beef, chili, onion, cucumber, tomato, lime juice, chilli, garlic	grill (beef) no heat treatment (vegetables)
B	khâaw phat (Fried Rice)	rice with meat and vegetables	stir fry
	phat thay (Thai stirred noodle)	noodles, meat, egg, tamarind juice, tofu, lime juice, bean sprout	stir fry
	laap (Thai minced meat with herbs and spices)	minced meat, mint, onions, coriander, ground roasted rice, chilli, lime juice, garlic	stir fry
C	tôm yam (Thai Soup)	meat, mushroom, tomatoes, lemongrass, chilli, lime juice	boiling
	tôm khâa (Thai Soup)	meat, mushroom, tomato, coconut milk, chilli, lemongrass, galangal, lime juice	boiling
	kɛɛn khĭaw wăan (Thai green curry)	meat, vegetable, coconut milk, green thai curry sauce, chilli	boiling
	kɛɛn phèt (Thai red curry)	meat, vegetable, coconut milk, red thai curry sauce, chilli	boiling

From the 19 restaurants, one was excluded because it did not offer a take-away option for the foods. Sampling was done in two rounds (summer, i. e. May through August, and winter, i. e. November through December), attempting to obtain one sample per food category "A"–"C" and restaurant and round, resulting in a total of 108 samples. In addition, 20 rice samples (Category "D") and 5 vegetable samples (Category "E") were collected. The overall number of samples was 133. Samples, obtained as full portions in take-away packages, were transported in a refrigerated box and arrived in the laboratory within 60 min, where they were analyzed without delay.

Sample examination

Upon arrival, samples were weighed and then subjected to microbiological testing. In brief, the entire portion was homogenized under sterile conditions, and from 25 g aliquots, serial tenfold dilutions were prepared in 0.1 % peptone water (Oxoid CM0733) and aliquots spread onto selective agars to assess total aerobic count (TAC), and numbers of *E. coli,* Enterobacteriaceae, *Enterococcus* spp., *Pseudomonas* spp., *Staphylococcus aureus, Bacillus cereus* and *Clostridium perfringens* (Table 2). In addition, sample aliquots of 25 g were tested for the presence of *E. coli, Salmonella* spp., *Listeria monocytogenes* and *Campylobacter* spp. (Table 3). Reference values for microbiological condition of ready-to-eat foods were adopted from the German Society for Hygiene and Microbiology (DGHM, 2012); different reference values for meals served hot and cold were considered.

In the remaining sample, pH was measured by a penetrating electrode (Schott blue line on a CG820 pH-meter, Schott, Germany).

Statistical analysis

Weight and pH of dishes were analyzed by one-way ANOVA and Fisher's LSD to discriminate among means, with food categorization as independent variable (SPSS 17.0; SPSS, US). For quantitative microbiological analysis, results below the limit of detection were set to: limit of detection minus 2; and results exceeding the detection range were set to: upper limit plus 1. Values were log transformed and tested by two-way ANOVA, with food categorization and sampling season as independent variables. Results from qualitative microbiological tests were assessed by chi-square tests. Statistical significance was established at $P = 0.05$ or 0.01.

TABLE 2: *Media and procedures used for quantitative microbiological analysis.*

Microorganism	Media and procedure
TAC	Colony counting on plate count agar, incubation 30 °C, 72 h.
Enterobacteriaceae	VRBD Agar (Merck 1.10275.0500) incubation 37 °C, 24 h, anaerobic condition.
Pseudomonas spp.	GSP-Agar (Pseudomonas-Aeromonas) (MERCK Nr. 1.10230.0500) incubation at room temperature (approx. 25 °C), 48 h.
E. coli	Coli ID agar (Biomerieux 42017) incubation 42 °C, 24 h, aerobic condition.
Enterococcus spp.	Chromocult Enterococci Agar (Merck Nr. 1.00950.500), incubation 42 °C, 48 h, aerobic condition.
Clostridium perfringens	Perfringens agar (OPSP) (OXOID CM0543) incubation 42 °C, 48 h, anaerobic condition.
Bacillus cereus	Bacillus cereus agar base (OXOID CM617) incubation 37 °C, 24 h, aerobic condition.
Coagulase-positive *Staphylococcus aureus*	Baird Parker RPF agar agar (BioMerieux 43531) incubation 37 °C, 48 h, aerobic condition.

TABLE 3: *Media and procedures used for detection of E. coli, Salmonella spp., Campylobacter spp. and Listeria monocytogenes.*

Microorganism	Media and procedure
E. coli	Enrichment in 225 ml Buffered Peptone Water (OXOID CM0509), incubation 37 °C, 24 h; streaking onto Coli ID agar (Biomerieux 42017) incubation 42 °C, 24 h, aerobic condition.
Salmonella spp.	Enrichment in 225 ml Buffered Peptone Water (OXOID CM0509), incubation 37 °C, 24 h; inoculation onto the MSRV motility agar (OXOID CM0910) incubation 42 °C, 24h; streaking suspected sample onto XLD-agar (Merck Nr.1.05287), incubation 37 °C, 24h; testing the colonies with typical morphology by agglutination (polyvalent I serum, Dade Behring).
Campylobacter spp.	Enrichment in 225 ml Bolton broth (OXOID CM0983, supplement: OXOID SR0208E), incubation 42 °C, 48 h microaerobic condition. Modified charcoal-cefoperazone-deoxycholate agar (mCCDA; CM0739) with supplement (SR155E, Oxoid) 42 °C for 48 h under microaerobic conditions (10 % CO_2, 5 % O_2, and 85 % N_2)
Listeria monocytogenes	Enrichment in 225 ml ½ Fraser Broth [Fraser Broth Base (OXOID CM0895), Fraser Listeria selective supplement (Merck Nr.1.00093), 1.0 g/l Ammonium-ferric(III)-citrate (MERCK 3762)] incubation 30 °C, 48 h aerobic condition. Enrichment culture is streaked onto ALOA Agar (OXOID CM1084, supplements SR0228E and SR0227E); incubation at 37 °C, 48 h aerobic condition.

Results and Discussion

Physical and chemical characteristics of Thai foods

The average weight of dishes of category "B" (presumably moderate risk) was 506 g, and, thus, significantly higher than that for categories "A" (high risk) and "C" (low risk). However, there is a considerable variation (factors ranging from 2 to >3) in serving sizes per category. Portions of side dishes ("D","E") were, expectedly, smaller than those of main dishes (Table 4).

TABLE 4: *Serving sizes (g) and pH in Thai foods in Viennese restaurants.*

Category	n	Serving size (g)		pH	
		mean ± std.dev.	min–max	mean ± std.dev.	range
A	36	363.0 ± 98.3[a]	210.4–589.7	4.47 ± 0.49[a]	2.93–5.14
B	35	506.2 ± 134.9[b]	217.1–723.9	5.37 ± 0.47[b]	4.68–6.32
C	37	419.6 ± 119.1[a]	198.2–740.5	5.74 ± 0.53[c]	3.92–6.49
D	20	255.6 ± 81.5[c]	116.6–473.4	6.55 ± 0.26[d]	6.08–7.31
E	5	122.4 ± 77.5[c]	52.4–253.2	5.96 ± 0.61[b,c,d]	5.17–6.64

Within columns, means with different superscript letters indicate significant differences.

As shown in Table 4, the pH of Thai foods ranged from 2.93 to 7.31, with lowest pH in category "A". Raw or uncooked dishes (category A; presumably high risk) of Thai food usually have an extreme taste such as a spicy taste (chili and garlic) or a sour taste (lime juice and tomato) that may lower the pH value down to ca. 3.0. As this low pH is generally not suitable for growth of the microbial agents of concern, it could be considered as a protective factor against pathogenic as well as spoilage bacteria in food. In contrast, Thai dishes in category C (cooked dishes; presumably low risk) are usually similar to Chinese food in terms of ingredients and cooking methods. As expected, the pH value of dishes in this category (5.74 ± 0.53) was lower than those found in Chinese food by Catellani et al. (2010), who report average pH of the Chinese first and second course as 6.91 and 6.49, respectively, corresponding to the more sour taste of Thai foods.

Overall microbiological condition of Thai foods, main dishes (categories "A" to "C")

All samples were negative for the major pathogenic bacteria *Salmonella* spp., *Listeria monocytogenes* and *Campylobacter* spp. in 25 g aliquots. Among the main dishes, foods from category "C" were characterized by lower bacterial numbers as regards TAC, *Pseudomonas* and *Enterococcus* (Table 5).

These results are similar to those reported by Catellani et al. (2010) who studied Chinese food from restaurants and take-away premises in Italy, and detected no *Salmonella* spp. and *Listeria monocytogenes* in 118 samples. In contrast, studies in Thailand reported a prevalence of *Salmonella* spp. in ready-to-eat-food of 2.0–4.3 % (Chomvarin et al., 2006; Teague et al., 2010). *Campylobacter* were, however, not detected in these Thai studies. The total number of samples was not sufficient to conduct an in-depth analysis whether or not spices would have a reducing effect on bacterial number.

Microbiological condition of dishes without, or having undergone unclear heat treatment (category "A")

TAC ranged from 4.5 to 7.9 log cfu/g, with a mean value of 6.1 ± 0.8 log cfu/g. Enterobacteriaceae, *Pseudomonas* and *Enterococcus* were the major groups of bacteria found. Enterobacteriaceae ranged from <2 to 7.0 log cfu/g, with a median of 3.0 log cfu/g. *Pseudomonas* and *Enterococcus* were found in the range of <2 to 7.3 log cfu/g, with a median of 4.9 log cfu/g and <2 to 7.2 log cfu/g with a median of 2.3 log cfu/g, respectively (see Table 5). *E. coli* was recovered in 25 g aliquots from 15 of 36 samples (41.7 %) and only in 3 samples (8.3 %) the 2 log cfu/g limit for *E. coli* was exceeded.

Pathogenic bacteria were found in few samples and generally in low numbers. *Staphylococcus aureus* and *Bacillus cereus* were detected in only 2 samples (5.6 %). *Clostridium perfringens* was detected in 8 samples (22.2 %), with a range from 1 to 3.3 log cfu/g.

TABLE 5: *Microbiology results of Thai dishes in log cfu/g.*

Microorganism	Category	Mean ± std.dev.	n	Number of samples (log cfu/g)						
				<2	2–3	3–4	4–5	5–6	6–7	7–8
TAC	A	6.1 ± 0.8	36	3	7	4	3	12	15	6
	B	4.5 ± 1.7	35	11	17	7	5	5	9	2
	C	2.6 ± 0.9	37					1	1	
Enterobacteriaceae	A	3.3 ± 1.3	36	11	5	10	6	2	1	1
	B	2.8 ± 1.2	35	18	5	4	4	4		
	C	<2	37	37						
E. coli	A	2.0 ± 0.2	36	33	2	1				
	B	1.9 ± 0.0	35	34	1					
	C	<2	37	37						
Pseudomonas spp.	A	4.7 ± 1.7	36	7	1	5	6	5	11	2
	B	3.0 ± 1.4	35	20	1	1	8	3	1	1
	C	2.0 ± 0.3	37	35		1				
Enterococcus spp.	A	2.6 ± 1.0	36	14	14	6	1	1	1	
	B	2.4 ± 0.8	35	22	6	3	3	1		
	C	2.1 ± 0.6	37	36						
Coagulase-positive *Staphylococcus aureus*	A	2.0 ± 0.1	36	34	2					
	B	<2	35	34	1					
	C	<2	37	37						
Bacillus cereus	A	1.9 ± 0.0	36	34	2					
	B	2.0 ± 0.0	35	33	2					
	C	2.0 ± 0.1	37	34	2	1				
Clostridium perfringens	A	1.2 ± 0.6	36	32	1	3				
	B	<1	35	35						
	C	<1	37	37						

A: dishes without or unclear heat treatment; B: dishes with raw material added to already cooked dishes; C: dishes with presumably "safe" heat treatment served alone

The results of this study show both lower prevalence and lower concentration of *Staphylococcus aureus* as compared with the findings of Chomvarin et al. (2006), who found this bacterium in 12.6 % of unheated and low-heated Thai food, and in concentrations of 2 to >5 log cfu/g. Likewise, Piyasirananda and Boriboon (2006) reported higher prevalences of pathogenic and hygiene indicator bacteria such as *Clostridium perfringens* (5.7 %), *Staphylococcus aureus* (4.3 %), *Salmonella* (2.2 %) and *E. coli* (25.1 %) in ready-to-eat papaya salad. Suspected sources of contamination were fermented fish and fermented whole crab.

Microbiological condition of meals with raw food components added to cooked dishes ("B")

TAC ranged from <2 to 7.6 log cfu/g, with a mean value of 4.5 ± 1.7 log cfu/g. Enterobacteriaceae, *Pseudomonas* and *Enterococcus* were major groups of bacteria found. Enterobacteriaceae counts were <2 log cfu/g for 18 out of 35 samples, and in the remaining samples, maximum content was 5.6 log cfu/g. *Pseudomonas* and *Enterococcus* were found in the range of <2 to 7.0 log cfu/g and <2 to 5.9 log cfu/g, respectively, with 20 and 22 out of 35 samples found <2 log cfu/g, respectively. *E. coli* in 25 g aliquots was recovered from 6 of 37 samples (17.1 %) and only 1 sample (2.9 %) exceeded the 2 log cfu/g limit for *E. coli,* as shown in Table 5.

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Pathogenic bacteria were hardly found. *Staphylococcus aureus* was detected in 1 sample (2.9 %) exceeding the warning limit of 2 log cfu/g. For *Bacillus cereus,* only 2 samples (5.7 %) were above the limit of detection (2 log cfu/g). *Clostridium perfringens* was detected in one sample at 1.9 log cfu/g.

Microbiological condition of dishes with heat treatment ("C")

TAC ranged from <2 to 6.1 log cfu/g, with a mean value of 2.6 ± 0.9 log cfu/g. In all samples, Enterobacteriaceae and *E. coli* numbers were below the detection limit at 2 log cfu/g. *E. coli* was detected only 1 out of 37 samples (2.7 %) in 25 g aliquots. *Pseudomonas* above detection limit was found in only 2 samples (5.4 %), with a maximum of 3.8 log cfu/g, while *Enterococcus* was found in only 1 sample (2.7 %) at 5.8 log cfu/g. *Bacillus cereus* was the only pathogenic bacterium found above the detection limit (3 samples, or 8.1 %).

These findings concur with those of Catellani et al. (2010), who reported that the majority (60 %) of dishes that had undergone a strong and fast heat treatment before being served had TAC values ≤3 log cfu/g. With respect to *Staphyloccus aureus,* a Thai study (Chomvarin et al., 2006) reported 3.5 % positive samples among high-heat treated foods. This is more than we found in Thai food samples in Vienna. One possible explanation is the sampling site; the Thai study included food from street vendors, where improper storage of heat treated food might have favored either the contamination with these bacteria or the growth of surviving Staphylococci, whereas the study in Vienna considered only food freshly prepared on consumer demand.

Relation of the microbiological condition of Thai main dishes to food categorization

TACs among Thai foods from the three categories "A"–"C" were significantly different (ANOVA; P <0.01). As expected, dishes with presumably safe heat treatment ("C") had lowest TACs followed by cooked dishes to which raw vegetables had been added after the end of cooking process ("B") and dishes without heat treatment ("A"). Likewise, there was an association between food categories and presence of *E. coli* in 25 g food aliquots, with foods of category "A" having a significantly higher chance of being *E. coli* positive (Pearson chi square; P <0.05), or having *E. coli* number above the limit of detection (Table 5). These findings are in line with the observation of Chomvarin et al. (2006). As the pathogenic bacteria tested in this study were either not detectable or only present at low levels, it is not possible to elucidate if the three presumptive risk categories would really have different microbiological profiles as regards pathogenic bacteria, but – with respect to hygiene indicators, as *E. coli* and Enterobacteriaceae – there is a clear difference between categories "C" and "A", "B". This could mean that the presumptive categories "A" and "B" could be merged. Further studies on this issue are currently being conducted.

Relation of the microbiological condition of Thai main dishes to recommended microbiological limits for ready-to-eat foods

The microbiological condition of the samples we studied were compared to warning limits recommended by the German Society for Hygiene and Microbiology (DGHM, 2012) (Table 6). For group "A" foods, warning limits for TAC, Enterobacteriaceae, *E. coli* and *Clostridium perfringens* were exceeded in 58.3 %, 30.6 %, 8.3 % and 8.3 % of samples, respectively, whereas in Group C, the majority of samples were below the warning limits. Group B had a somewhat intermediate position.

TABLE 6: *Number of samples of Thai main dishes exceeding warning limits recommended by German Society for Hygiene and Microbiology (DGHM, 2012).*

Microorganism	A n = 36 (%)	B n = 35 (%)	C n = 37 (%)	Warning limits (cfu/g)
TAC	21 (58.3)	21 (60.0)	2 (5.4)	1×10^6 (Cat. B, C) 1×10^4 (Cat. A)
Enterobacteriaceae	11 (30.6)	13 (37.1)	0 (0)	5×10^3 (Cat. B, C) 5×10^2 (Cat. A)
E. coli	3 (8.3)	1 (2.9)	0 (0)	1×10^2
Coagulase-positive Staphylococci	0 (0)	1 (2.9)	0 (0)	1×10^3 (Cat. B, C) 1×10^2 (Cat. A)
Bacillus cereus	0 (0)	0 (0)	1 (2.7)	1×10^3
Clostridium perfringens	3 (8.3)	0 (0)	0 (0)	1×10^3

A: dishes without or unclear heat treatment; B: dishes with raw material added to already cooked dishes; C: dishes with presumably "safe" heat treatment served alone note that different warning limit apply according to heat treatment, see last column

Seasonal effect on microbiological condition of Thai foods

A two-way ANOVA was conducted to examine the effect of season and food categorization ("A"–"C") on TAC. There was no significant interaction between the effects of season and heat treatments on TAC (P = 0.693). Heat treatment exerts the main effect (P <0.01), while season shows no effect on TAC (P = 0.567). Likewise, within categories, TAC of Thai main dishes sampled during summer and winter season were not statistically different.

Microbiological condition of side dishes

Salmonella spp., *Listeria monocytogenes, Campylobacter* spp., *Bacillus cereus* and *Clostridium perfringens,* were below the detection limit in all 25 samples.

In rice samples, TAC ranged from <2 to 6.3 log cfu/g, with a mean value of 2.6 ± 1.3 log cfu/g (Table 7). TAC exceeded the DGHM (2012) warning limit in 3 samples (15 %). Only in 1 out of 20 samples (5 %) Enterobacteriaceae were above the warning limit (3.3 log cfu/g) (Table 8) and 2 out of 20 (10 %) were positive for *E. coli* from a 25 g aliquot. *Pseudomonas* was detected in 4 (20 %) samples and represented the major bacterium found in rice samples, ranging from <2 to 5.1 log cfu/g, with a median <2 log cfu/g. *Enterococcus* and *Staphylococcus aureus* were below the detection limit in all 20 samples.

As rice is usually completely heated during cooking, the microbial contamination indicates improper hygiene and handling except for *Bacillus cereus* which is a spore-forming bacterium tolerant to heat, and most commonly found in re-heated rice (Gibbs, 2002).

In the five vegetable samples, TAC ranged from 3.9 to 6.6 log cfu/g, with a mean value of 4.9 ± 1.2 log cfu/g. TAC exceeded the warning limit in one sample only. Enterobacteriaceae were detected in 4 of 5 samples but only 1 sample (3.0 log cfu/g) was above the warning limit (Table 8). Only one sample was positive for *E. coli* in a 25 g aliquot. *Pseudomonas* counts ranged from 2.4 to 6.6 log cfu/g, with mean value of 4.3 ± 1.5 log cfu/g. *Enterococcus* and *Staphylococcus aureus* were detected in 1 sample, with a count of 2.4 log cfu/g and 2.6 log cfu/g, respectively.

As compared to the study of Abadias et al. (2008) – reporting TACs of minimally processed (fresh-cut) vegetable

TABLE 7: *Microbiological condition of side dishes; rice ("D") and fresh vegetable ("E") in log cfu/g..*

Microorganism	Category	Mean ± std.dev.	n	Number of samples (log cfu/g) <2	2–3	3–4	4–5	5–6	6–7
TAC	D	2.6 ± 1.3	20	12	4	1	1	2	1
		4.9 ± 1.2	5			2		1	1
Enterobacteriaceae	D	2.0 ± 0.2	20	19		1			
		2.4 ± 0.4	5	1	3	1			
Pseudomonas spp.	D	2.3 ± 0.8	20	16	1	1	1	1	
		4.3 ± 1.5	5		1	1	2		1
Enterococcus spp.	D	2.1 ± 0.8	20	19				1	
		2.0 ± 0.2	5	4	1				
Coagulase-positive	D	<2	20	20					
Staphylococcus aureus	E	2.1 ± 0.2	5	4	1				

TABLE 8: *Number of samples exceeding microbiological warning limits for rice and vegetable (DGHM, 2012).*

Microorganism	D n = 20 (%)	E n = 5 (%)	Warning limits (cfu/g)
TAC	3 (15)	1 (20)	1 x 10^6 (Cat. E) 1 x 10^4 (Cat. D)
Enterobacteriaceae	1 (5)	0 (0)	5 x 10^3 (Cat. E) 5 x 10^2 (Cat. D)
E. coli	0 (0)	0 (0)	1 x 10^2
Coagulase-positive Staphylococci	0 (0)	0 (0)	1 x 10^3 (Cat. E) 1 x 10^2 (Cat. D)
Bacillus cereus	0 (0)	0 (0)	1 x 10^3
Clostridium perfringens	0 (0)	0 (0)	1 x 10^3

D: rice (heat treated); E: fresh vegetable (raw) as side dishes; note that different warning limit apply according to heat treatment, see last column

ranging from 4.3–8.9 log cfu/g, with mean value of 7.0 log cfu/g, a mean value of 3.5 log cfu/g for Enterobacteriaceae, and 11.4 % positive for *E. coli* and 1.7 % positive for *Salmonella* – the values found in the present study are lower, but the very limited number of samples should be taken into account.

Conclusions

In this study, an approach is described how a particular group of ethnic foods could be classified according to heat treatment and the likelihood of cross-contamination. This empirical classification was intended to characterize the risk that pathogenic bacteria are present in the ready-to-eat food portion. This classification scheme was well reflected in total aerobic counts. With respect to hygiene indicators, there was a clear association between presumably insufficient heat treatment and/or the likelihood of cross-contamination and the presence of *E. coli* and Enterobacteriaceae. As regards pathogens, there was no clear relation between detection of pathogens and food classification, most probably due to the low frequency of pathogens. This prompts for conducting further studies on this issue.

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